Physiological characteristics of the Keap1-Nrf2 system in zebrafish

著者（英）	Nguyen Thanh Vu
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氏　　名	Nguyen Thanh Vu
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主　　査	筑波大学教授	博士（理学）	入江　賢児
副　　査	筑波大学教授	医学博士	加藤　光保
副　　査	筑波大学准教授	博士（医学）	工藤　崇
副　　査	筑波大学准教授	博士（農学）	中川　嘉

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Nguyen Thanh Vu describes the physiological characteristics of the Keap1-Nrf2 system in zebrafish. The summary is as follows:

（**目的**　Purpose）

The Keap1-Nrf2 system plays an important role in protecting animals from environmental stresses and its functions and regulatory mechanism are highly conserved among vertebrates. Nrf2 is a transcription factor that regulates anti-oxidative stress proteins and detoxification enzymes. Keap1 is a cytosolic protein that interacts with Nrf2 to promote its ubiquitination and proteasomal degradation. Interestingly, Keap1 can also sense a variety of stress and chemicals and changes its protein conformation to disrupt the proper Keap1-Nrf2 interaction for the ubiquitination, leading to stabilization of Nrf2 and transactivation of its target genes. Although the regulatory mechanism and functions of the Keap1-Nrf2 system have been extensively studied, many aspects of the Keap1-Nrf2 system are still not well understood; e.g., physiological roles at animal level; the molecular basis of stress/chemical sensing. In this study, the author aimed to discover and clarify the new regulatory mechanism and functions of the Keap1-Nrf2 system.

（**対象と方法**　Materials and Methods）

To elucidate new aspects of the Keap1-Nrf2 system, the author used zebrafish as a model animal. In the Kobayashi laboratory to which the author belongs, research of the Keap1-Nrf2 system using zebrafish has been carried out for many years and materials and methods for studying this system have been established, including

zebrafish mutant lines, such as *nrf2a*^{fh318} and *pmm2*^{it768}; gene expression analysis using whole-mount in situ hybridization and real-time qPCR; knockdown analysis using morpholino oligonucleotide; and gene knockout analysis by CRISPR-Cas9.

（**結果**　Results）

The author did the following four research projects.

1) Searching the new target genes of zebrafish Nrf2

In microarray analysis, the applicant analyzed genes which were found to be upregulated by overexpressing zebrafish Nrf2. Consequently, the applicant found that the genes encoding proteasome subunits and glucose metabolism enzymes were upregulated by zebrafish Nrf2.

2) Elucidation of regulatory mechanism and functions of the Nrf2 activation in zebrafish *pmm2*^{it768} mutant which has defects in *N*-glycosylation.

The author found that ER stress was induced in *pmm2*^{it768} larvae and it activated the Keap1-Nrf2 system. In order to understand the physiological contribution of Nrf2 to the ER stress response, the author generated *pmm2*^{it768};*nrf2a*^{fh318} compound mutants. Nrf2 activating photochemical sulforaphane could attenuate the induced ER stress in *pmm2*^{it768} single mutants, but not in *pmm2*^{it768};*nrf2a*^{fh318} compound mutants, indicating that ER stress was downregulated by the Keap1-Nrf2 system.

3) Generation and characterization of *keap1a* and *keap1b* knockout zebrafish.

Zebrafish has two Keap1 genes, *keap1a* and *keap1b*. *keap1b* is a homology of mammalian Keap1 and *keap1a* is a fish and amphibian specific Keap1. To understand the physiological roles of *keap1a* and *keap1b,* the keap*1a* and *keap1b* knockout zebrafish were generated by CRISPR-Cas9 method. Both *keap1a* and *keap1b* knockout zebrafish were viable and fertile. Nrf2 was upregulated in both *keap1a* and *keap1b* knockout zebrafish larvae, and those larvae were stronger against oxidative stress than wild-type. Interestingly, *keap1a* knockout larvae respond more to sulforaphane than *keap1b* knockout zebrafish larvae, suggesting that keap1b protein is a good sensor for sulforaphane than keap1a protein.

4) Characterizations of *keap1a;keap1b* double knockout zebrafish.

keap1a;keap1b double knockout zebrafish was lethal at age of 9 days larvae.

（**考察**　Discussion）

The author discusses that there are three interesting findings in this study:

1) The genes encoding proteasome subunits and glucose metabolism enzymes have been identified as new target genes for zebrafish Nrf2, suggesting that protein and glucose metabolism can be regulated by the Keap1-Nrf2 system in fish as well as in mammals.

2) The Keap1-Nrf2 system responds to ER stress and attenuated this cellular stress, implying lifestyle disease caused by ER stress can be prevented by talking Nrf2-activation foods/drinks.

3) Zebrafish keap1a showed similar functions and activities with keap1b and mammalian Keap1, except for sulforaphane sensing. It is interesting to identify the protein structures of Keap1s that reflect this similarity and difference.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

In the present study, the author has identified the new target genes of zebrafish Nrf2. The author also revealed that the Keap1-Nrf2 system responds to ER stress and attenuated this cellular stress. The author generated and characterized *keap1a* and *keap1b* knockout zebrafish. The findings in this study will surely have impact in the field and will benefit for understanding of physiological roles of the Keap1-Nrf2 system.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on 29 May, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.